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Materials

Jingwei Xie

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An abbreviated version of this protocol was published in Science Advances in Jul 2021

Biomaterials with structural hierarchy and controlled 3D nanotopography guide endogenous bone regeneration

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Detailed protocol

BMSCs migration

The Gel-MA was sterilized with ethylene oxide for 12 h. Then, 8% (w/v) of Gel-MA solution with 0.05% (w/v) 2959 photoinitiator was prepared in 0.01M PBS buffer (pH 7.4). And 3 ml of the mixed solution was added into each well of the 6-well plate and crosslinked with UV light for 1 min. The Gel-MA hydrogels were washed with 0.01 M PBS for 3 times before use. BMSCs were isolated from Sprague–Dawley rats (male, 6-month old) following standard procedures. The fourth-generation of BMSCs was used for the migration study. One milliliter of BMSCs solution (1×10^7 cells/ml) was seeded on the surface of 8% Gel-MA hydrogel and cultured until the cell confluence reached 90%. Two different in vitro migration models were established. In the first model, the RAS or VAS were placed on the surface of BMSCs seeded Gel-MA hydrogel and continuously cultured for 2, 4, and 6 days. In the second model, an 8-mm hole was created by a punch, then RAS or VAS were inserted into the hole. The surface of scaffolds and the surface of hydrogel were kept at the same level, and then continuously cultured for 4, 8, and 12 days.

Imaging

The BMSCs seeded on the surface of Gel-MA hydrogel without or with an 8-mm hole were fixed with 4% paraformaldehyde and permeabilized with a 0.1% Triton X-100 solution in PBS. Subsequently, the BMSCs were stained with Alexa Fluor™ 546 Phalloidin (dilution 1:200) for 20 min. Then, the BMSCs seeded on the surface of Gel-MA hydrogel without or with an 8-mm hole were imaged by confocal laser scanning microscopy (CLSM) (Zeiss 880, Oberkochen, Germany). The z-stack range was set from 0 μm to 250 μm, the interval was set at 10 μm, and the tile scans were set as 2×2 for hydrogel without a hole, and 7×7 for hydrogel with an 8-mm hole. At each indicated time point, the BMSCs infiltrated RAS and VAS were collected and fixed with 4% paraformaldehyde and permeabilized with a 0.1% Triton X-100 solution in PBS. Then, the scaffolds were stained with Alexa Fluor™ 546 Phalloidin (dilution 1:400) for 20 min. Finally, the RAS and VAS were imaged by CLSM. In the first migration model, the z-stack range was set from 0 μm to 1000 μm, and the interval was set at 10 μm. In the second migration model, the z-stack range was set from 0 μm to 900 μm, the interval was set at 10 μm, and the tile scan was

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Xie, J. (2021). Materials. Bio-protocol Preprint. bio-protocol.org/prep1362.
2. Chen, S., Wang, H., Mainardi, V. L., Talò, G., McCarthy, A., John, J. V., Teusink, M. J., Hong, L. and Xie, J.(2021). Biomaterials with structural hierarchy and controlled 3D nanotopography guide endogenous bone regeneration. Science Advances 7(31). DOI: 10.1126/sciadv.abg3089